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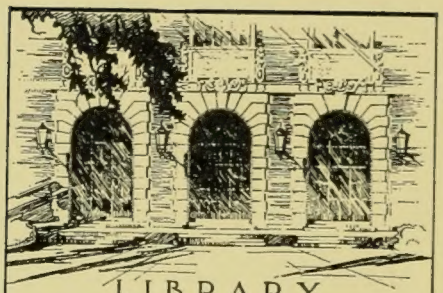

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CONTINUOUS MASS REARING OF THE EUROPEAN CORN BORER IN THE LABORATORY

Paul Surany

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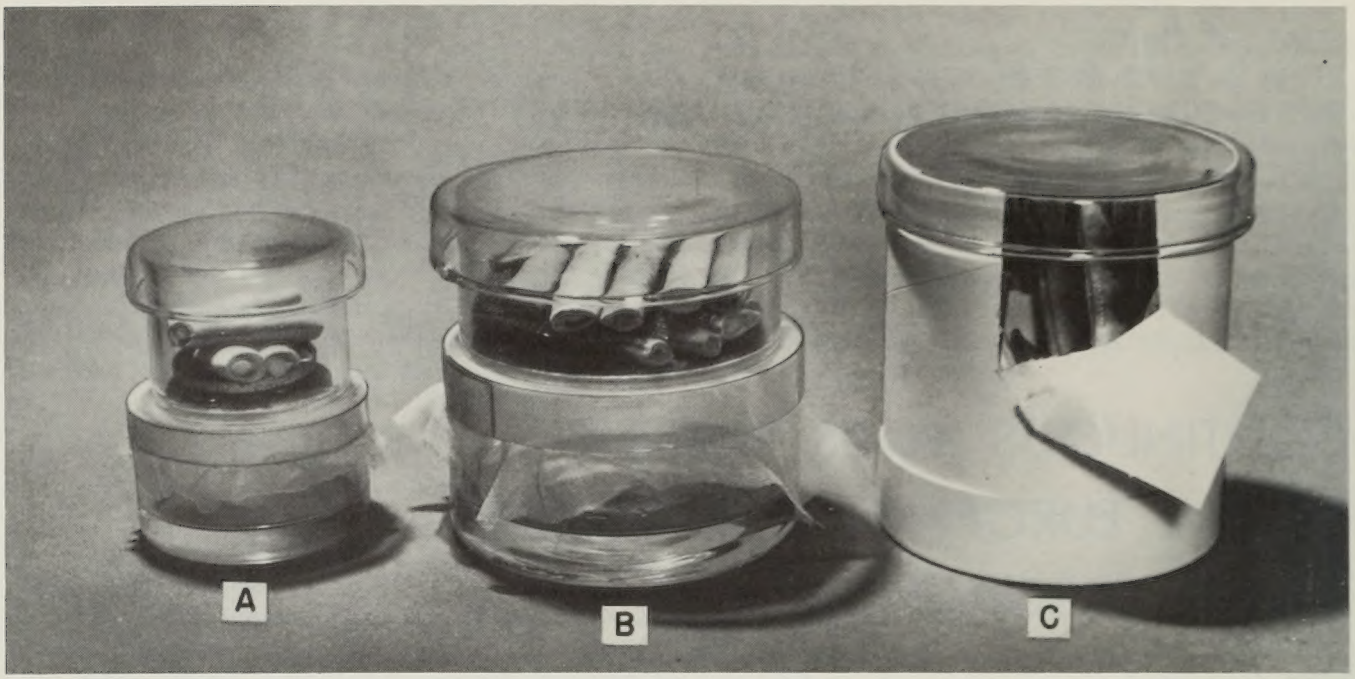

Fig. 1. – Three types of cages used in continuous mass rearing of European corn borer larvae: *A*, cage No. 1 with beans inside and with humidifier underneath; *B*, cage No. 2 with beans inside and with humidifier underneath; *C*, cage No. 3 with part of paper carton torn to show beans inside.

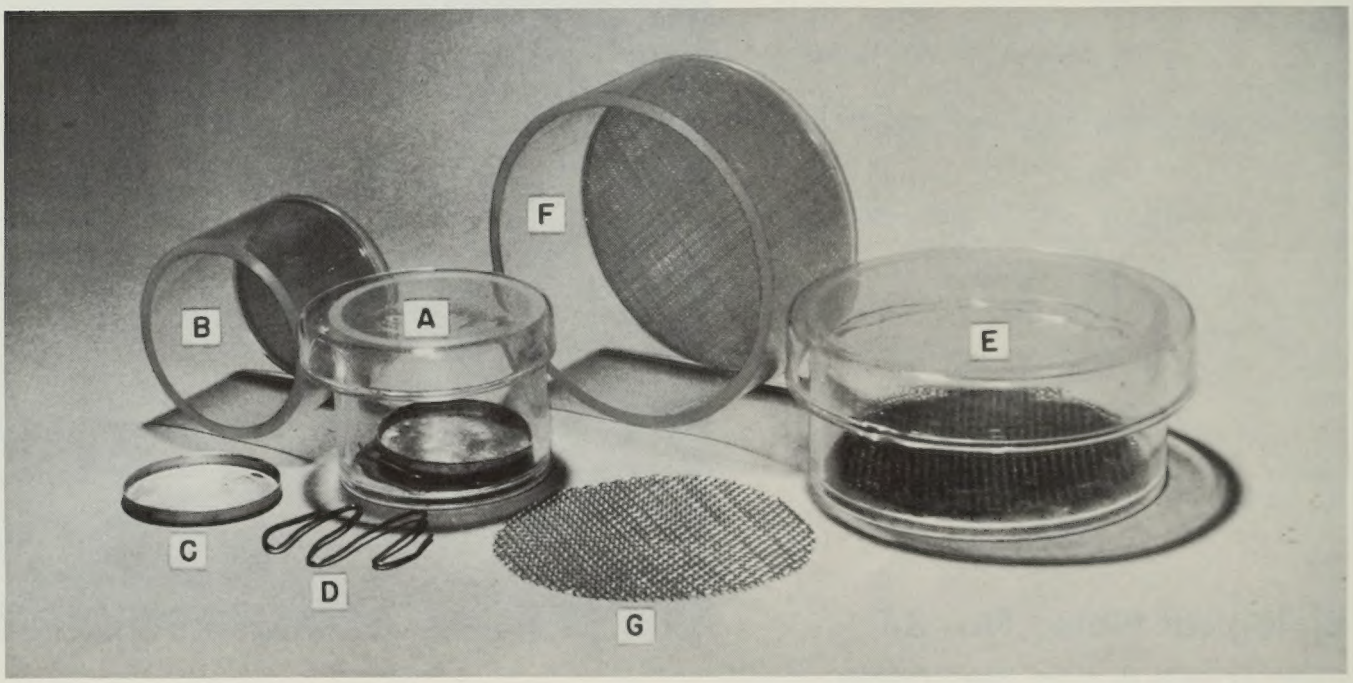

Fig. 2. – Cages No. 1 and No. 2 and their component parts. *A* is cage No. 1 complete, including top (top of 60-mm. petri dish), and *B*, *C*, *D* are component parts: *B*, 1-1/2-inch length of Lucite tubing (2 inches in diameter, one-eighth-inch wall thickness) with 80-mesh brass screen wire bottom; *C*, aluminum dish (32 mm. in diameter) to hold the beans; and, *D*, enameled copper wire in the form of a rough *M* or *W* to serve as a spacer beneath the aluminum dish. *E* is cage No. 2 complete, including top (top or bottom of 95-mm. petri dish), and *F*, *G* are component parts: *F*, 1-1/2-inch length of Lucite tubing (3-1/4 inches in diameter, one-eighth-inch wall thickness) with 50-mesh brass screen wire bottom and, *G*, one of two discs (2-3/4 inches in diameter) made from 18 x 14 mesh copper window-screen wire. (A layer of beans is laid between the two discs when the cage is in operation.)

CONTINUOUS MASS REARING of the European Corn Borer in the Laboratory

Paul Surany*

Several years ago, in the course of a research project designed to explore the possibilities of biological control of the European corn borer, *Pyrausta nubilalis* (Hbn.), there was an acute need for a large and steady supply of corn borer larvae in all instars.

The rearing of corn borer larvae in small numbers has been reported by a few workers. In laboratory tests involving several living plants, Mathes (1936) had reported that green beans followed by green peas constituted the most successful laboratory diet for corn borer larvae. In similar tests, Bottger (1940) had found that green beans, lettuce, and green peas, as well as some strains of corn, were satisfactory food plants. He had indicated that "food materials rich in glucose fulfilled the borer's nutritive requirements far better than did those high in either sucrose or starch." In nutrition studies of corn borer larvae, Bottger (1942) and Beck, Lilly, & Stauffer (1949) had successfully used synthetic culture media as food. The methods developed and described by these workers, although helpful, were inadequate to produce the large and continuous supply of larvae needed for the research project on biological control of the corn borer.

FOOD FOR CONTINUOUS MASS REARING

In the continuous mass rearing operations reported here, various culture media tried as food for the corn borer larvae proved to be unsatisfactory, and it was concluded that, under the prevailing laboratory conditions, only living or fresh plant material could meet the food requirements.

Corn, *Zea mays* L., the common host plant of the larvae, was considered unsatisfactory because an adequate supply of corn plants was not readily available throughout the year and because small pieces of the plant tended to wilt quickly, mold readily, and become unpalatable to the larvae.

After tests had been made of most of the fresh plant materials available throughout the year in grocery stores, the pods of string beans, *Phaseolus vulgaris* L., (either green or wax beans) were selected as the most satisfactory food for the corn borer reared under laboratory conditions. The larvae, first and later instars, accepted the fresh string bean pods without hesitation, and their attitude toward the pods did not differ much from their attitude toward pieces of the corn plant.

One of the chief advantages in using bean pods as food for corn borer larvae is in the fact that the pods usually do not decompose rapidly. On the surfaces cut with a sharp knife, the bean pieces quickly develop a callus tissue, which serves as a barrier against invasion of microorganisms. No unusual measures against contamination were necessary to prevent decomposition of the beans by molds and bacteria during a single phase of the rearing process.

EQUIPMENT FOR CONTINUOUS MASS REARING

In order to secure a large and continuous supply of corn borer larvae for the experiments in biological control, it became necessary to design and construct special equipment, principally containers in which the larvae could be reared.

One of the requirements for continuous mass rearing of corn borer larvae was a simple means of maintaining humidity in the rearing containers at or near the level optimal for the development of the larvae. The early instars require a humidity level near the saturation point which, unfortunately, is optimal for the development of molds and putrefying bacteria. The need of costly and complicated machinery for critical humidity control was overcome by scheduling each of three successive phases in the rearing of the larvae in a different type of container. In the interest of efficient work, cages of three different sizes were designed with the aim of providing each cage with its own physical atmosphere.

The cages constructed for continuous mass rearing of the larvae are illustrated in fig. 1. Cages No. 1 and No. 2 were made from Lucite or Plexiglass tubing, brass screen wire, and glass. Cage No. 3 was made from similar materials, except that a paper carton was substituted for the Lucite tubing.

For the smallest cage, No. 1, fig. 2*A*, a 1-5/8 inch length was cut with a band saw from tubing that was 2 inches in diameter and had a wall thickness of one-eighth inch. The edges of each length were finished square on a lathe to form the cylindrical wall of a cage 1-1/2 inches tall. For the bottom of the cage a disc was

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cut from an 80-mesh brass screen wire which was inserted with hand pressure into a groove on the inside of the tubing. The groove was cut on a lathe: it was 0.8 mm. wide, two-thirds of the tubing thickness in depth, one-eighth inch above the bottom edge of the cylinder in order to provide an air space beneath the screen. The screen was sealed into the groove by glacial acetic acid or other suitable solvent. The top of a standard petri dish 60 mm. in diameter provided the removable top for the cage.

The No. 2 cage, fig. 2*E*, was similar to the No. 1 cage, except that it was fashioned from Lucite tubing 3-1/4 inches in diameter (one-eighth inch wall thickness), and had a bottom of 50-mesh brass screen wire and a top that was either the top or the bottom of a standard petri dish 95 mm. in diameter. This cage, like the No. 1 cage, was 1-1/2 inches tall.

The cylindrical wall of the No. 3 cage, fig. 1*C*, was made from a pint-sized ice cream carton of heavy waxed paper or cardboard. The bottom and the top of the carton were removed, and the paper ring of the top was used to retain the screen bottom, which was 30-mesh brass screen wire. The top was either the top or bottom of a standard petri dish 95 mm. in diameter.

TECHNIQUE FOR CONTINUOUS MASS REARING

Rearing of the corn borer larvae was begun in No. 1 cages. The food for each No. 1 cage, two pieces of green string bean pods, each cut to a length of about 1-1/4 inches, was placed on an aluminum dish 32 mm. in diameter, fig. 2*C*. (This dish, also called a planchet, is the type used for radioactive assays.) A spacer, a 1 mm. diameter enameled copper wire in the form of a rough *M* or *W*, fig. 2*D*, was used between the pan and the screen bottom in order to facilitate the circulation of air and to prevent larvae from being crushed under the dish when the cage was moved. Two or three corn borer egg masses of normal size were placed on the beans in each cage, and the cage was covered with a petri dish top. If the top was slightly off center, freshly hatched borers wandering around in search of food were able to escape over the upper rim of the cage unless a filter paper disc of 55 mm. diameter was used as a liner inside the top. This liner, which insured a seal around the upper rim of the cage, was removed after the young borers had begun to feed.

The newly hatched larvae accepted the fresh green beans without hesitation and did not wander around for

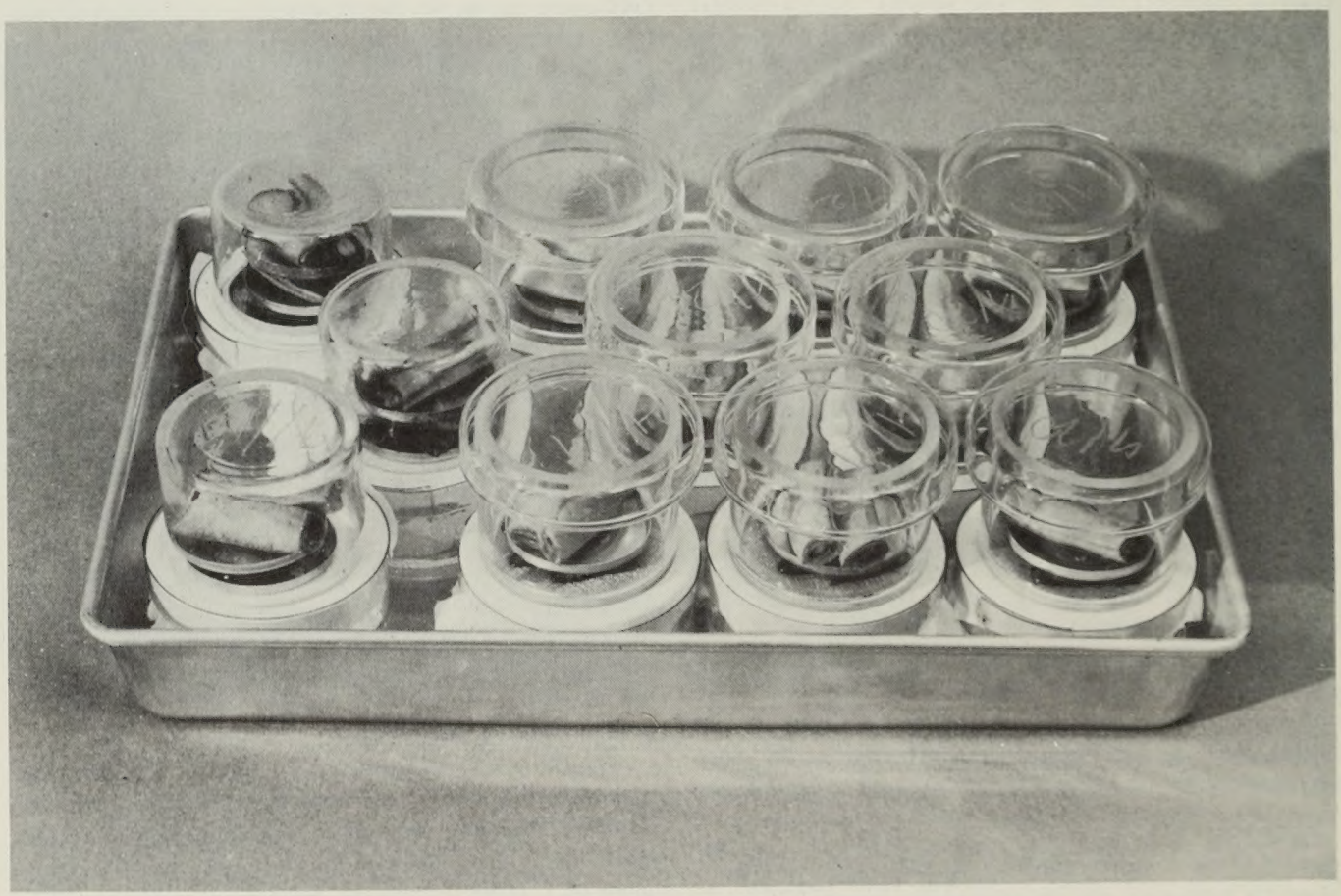

Fig. 3.—No. 1 cages. They contain corn borer larvae of first and second instars. Underneath each cage is a humidifier.

as long a time before feeding as they would normally on a corn leaf. They fed on the cut surfaces of the bean pods first and then they worked their way inward.

In order to maintain the high humidity favorable for development of the young borers, as well as to keep the beans fresh, each cage was placed on a dish partly filled with water, as shown in figs. 1*A* and 3. For use with each No. 1 cage, a standard crystallizing dish, 60 mm. in diameter, was covered with muslin, batiste, or organdy. The textile cover was kept taut by a celluloid ring on the outside perimeter of the dish. The water and the air space (3 mm.) beneath each cage helped to maintain the humidity in the cage at approximately 95 per cent. It was found that, in the course of routine rearing of mass cultures, there was no special need of keeping saturated salt solution in the dish below the cage. If the temperature was not subject to rapid changes, there was no perceptible accumulation of condensed moisture within the cage.

As the young borers developed, and a part of the food was consumed, a number of the borers appeared outside the bean pieces and began crawling around in the cages. At this time, two or three additional pieces of beans were placed in each cage. The borers accepted the fresh beans immediately, began to feed without hesitation, and ate their way into the beans. In about 2 additional days, most of the borers were in the second instar and appeared again crawling around in the cages in search of food. By this time all the larvae easily accessible were transferred with a wet camel's-hair brush to No. 2 cages. Each No. 1 cage was then placed on a tray covered with corrugated paper, where the food in the cage desiccated rapidly. In about 2 or 3 days all the remaining borers crawled out of the beans and were transferred without difficulty to No. 2 cages.

About 30 second or third instar larvae were placed in each No. 2 cage. Beans, cut to suitable lengths, were placed in a single layer on a screen disc, 2-3/4 inches in diameter, made of standard 18 x 14 mesh copper window-screen wire, and a similar disc was placed on the beans, fig. 1*B*. The second screen wire disc provided a place and spacer for the next layer of food and, at the time of transfer of larvae from the No. 1 cage to the No. 2 cage, was used to aid in stripping the larvae from the wet brush without injuring them.

The bottom screen disc in each No. 2 cage and the aluminum pan in each No. 1 cage helped to protect the permanent screen bottoms of the cages from the decaying beans at the end of a phase of rearing and, being removable, they also facilitated the cleaning of the cage.

It was usually necessary to keep each No. 2 cage for a few days above water to maintain high humidity in the cage. A crystallizing dish of 90 mm. diameter was the most suitable for this purpose. It was provided with a textile top, as described for the No. 1 cage. If it became evident a few days later that the humidity in the cage was too high, the cage was moved to a corrugated paper surface for slow desiccation of food, usually for as long as the cage was in use.

The time the corn borers had to be kept in the No. 2 cage was normally 4 to 6 days, by which time they had reached the late fourth or early fifth instar.

Fourth and fifth instar larvae seen crawling freely around in the cage were transferred with tweezers to the No. 3 cage. It was advisable to place a loose-fitting screen disc on the bottom of this cage before green string beans, broken in half, were thrown loosely into the cage. As the borers consumed a large quantity of food in the fifth instar, there was no special need to protect the food from dehydration. Therefore, the No. 3 cages were kept always on corrugated paper bases. To provide a suitable place for pupation, a corrugated paper strip (17 inches long, 1 inch wide) was placed around the inside wall of the cage about half way between the top and bottom of the cage, fig. 2*C*. If it did not stay in place, it was fastened with a short strip of adhesive tape.

The number of borers reared in each No. 3 cage was 15 to 20. Food was provided as long as needed. After the borers had pupated, the corrugated paper strip could either be removed and the pupae placed in oviposition cages, or the strip could be left in the No. 3 cage. In the latter case, the emerging moths clung to the undersurface of the cage top, from which they could be easily transferred to an oviposition cage.

Although successive transfers of the corn borer larvae from cage to cage required considerable labor, it seemed advisable to adopt this method rather than try to rear larvae to maturity in the same container. Complications resulting from excess humidity and from the inevitable putrefaction of the food after a certain period could thus be avoided, and the chances for cannibalism among larvae could be reduced.

EVALUATION OF CONTINUOUS MASS REARING

Observations indicated that European corn borers could be reared by the above-described method through a number of generations without any noticeable degeneration or harm to them. In laboratory experiments under controlled conditions, the larvae developed to the same size as did fifth instar larvae in the first generation of the bivalent race of the European corn borer in the field. They did not show a tendency to go into diapause. The moths may have been somewhat smaller in size than those that emerged from hibernating larvae collected in the field. The time necessary for development of the

corn borer larvae at three different temperatures is shown in the accompanying table.

Time needed for development (first instar-adult) of European corn borers reared on green bean pods in mass rearings.

Temperature	Days
70 degrees F.	26-38
80 degrees F.	17-25
90 degrees F.	15-17

The number of eggs produced by corn borer moths in the laboratory varied considerably. The females had a tendency to lay egg masses of the same sizes as those of females in the field.

For egg laying, the moths were kept in pint-size ice cream cartons. The top and bottom of each carton were removed and the bottom was replaced with a wire screen, which served as the top when the carton was inverted and placed on moist sand in a petri dish. A removable wax paper lining was placed in each carton, and on this lining the females laid their eggs.

The practice of keeping detailed records in the course of rearing operations proved to be very useful. The origin and the longevity of parent adults and the daily egg production were noted in relation to the fate and development of the progeny. The records or case histories could be used in the selection of strains from the most vigorous cultures. Also, they could be traced through a number of generations in the event of induced or accidental infections, or they could be consulted in nutritional or other studies.

SPECIAL REARING TECHNIQUES

Under certain circumstances, there was a need to rear a large number of European corn borer larvae for only a brief period for experiments of limited duration. Sometimes it was necessary to rear either newly hatched or fourth or fifth instar larvae in greater quantities than could be handled by the established methods.

Large numbers of newly hatched larvae could be reared with very satisfactory results in containers made from small paper cups (preferably souffle cups of 2-inch diameter) and petri dishes. Moisture did not condense within these containers. Three or four pieces of bean pod and an egg mass of normal size were placed in each cup, and the bottom part of a 55 mm. petri dish was placed on the top. The container was then inverted and a small quantity of melted paraffin was dropped around the inside rim of the bottom of the petri dish to provide a complete seal around the edge of the cup. Containers of this kind, with the glass tops up, were placed several together in a constant humidity chamber set at 90 per cent humidity, or as an alternative each was placed in a half-pint size ice cream carton together with a wad of wet cotton. The development of the borers could be observed through the glass tops.

If the borers were to be reared through several instars, a hole was cut in each paper cup soon after the larvae reached the second or third instar: this hole allowed larvae to leave the cup and feed on fresh beans placed loosely in the ice cream carton.

For screening tests, it was frequently necessary to have a large number of late first instar corn borer larvae. In such cases, the pieces of beans in which larvae were feeding were split carefully along the sutures and submerged in distilled water. A number of larvae would leave the food immediately and they could then be strained out of the water. The rest of the larvae could be forced to leave the beans by repeated application of low vacuum. The same method was used when there was a need for a large number of later instar larvae in a short time.

Frequently, a considerable number of fourth and fifth instar corn borer larvae were collected following the dissection of infested cornstalks in field experi-

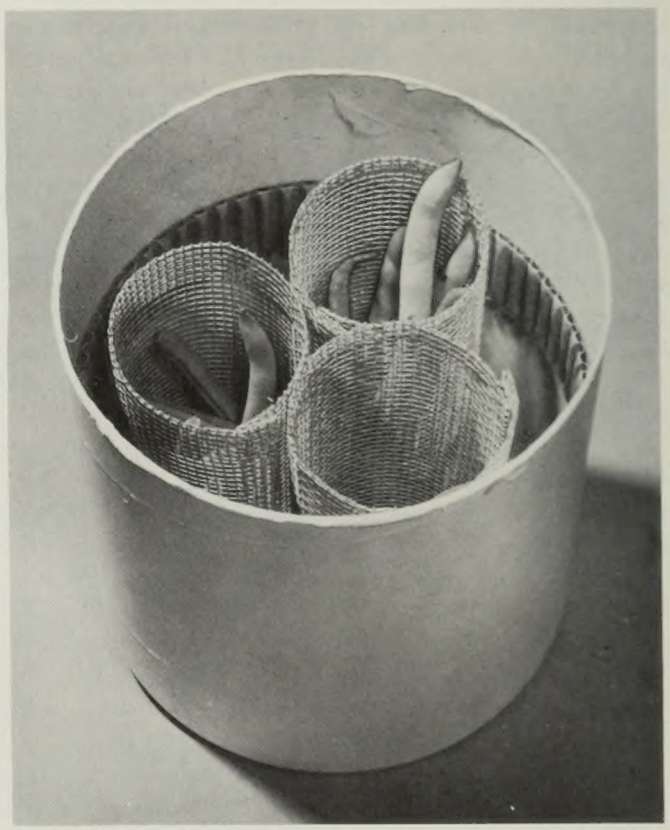

Fig. 4. — Arrangement for mass rearing of corn borer larvae of fourth and fifth instars. Cylindrical wire baskets containing food are placed, and replaced as the food is consumed by the larvae, in a 1-gallon ice cream carton. On the bottom of the carton is a layer of moist sand, and around the inside wall is a strip of corrugated paper to serve as a place for pupation.

ments. For mass rearing of these late instar larvae for a limited period, 1-gallon ice cream cartons proved to be very satisfactory, fig. 4. A layer of moist sand was placed in the bottom of each carton. A corrugated paper strip was taped around the wall to provide a place for pupation. The food, whole string bean pods, was placed in cylindrical wire baskets made from 8 x 8 mesh hardware cloth; each basket was 2-1/2 inches in diameter and 5-1/2 inches in length. The baskets were then placed on the sand in the cartons and as far as possible from the walls of the cartons. There was ample room for 40 to 60 larvae in each carton. The borers were dropped on the food, which they quickly accepted. As the food was consumed, additional baskets provided with food were placed in the cartons. The borers moved from basket to basket. The food was never in contact with the wall of a carton; therefore, borers seldom chewed holes in the wall of a carton and escaped.

For certain experiments that required the keeping of larvae under continuous observation and in individual confinement during their full development, shell vials of standard sizes were preferred as rearing containers. Use of the vials presented numerous problems, such as the condensation of moisture and the inadequacy of cotton or cork stoppers and plastic or aluminum caps, as well as suitable storage facilities for large numbers of vials in a manner that would render them individually accessible for easy observation and handling.

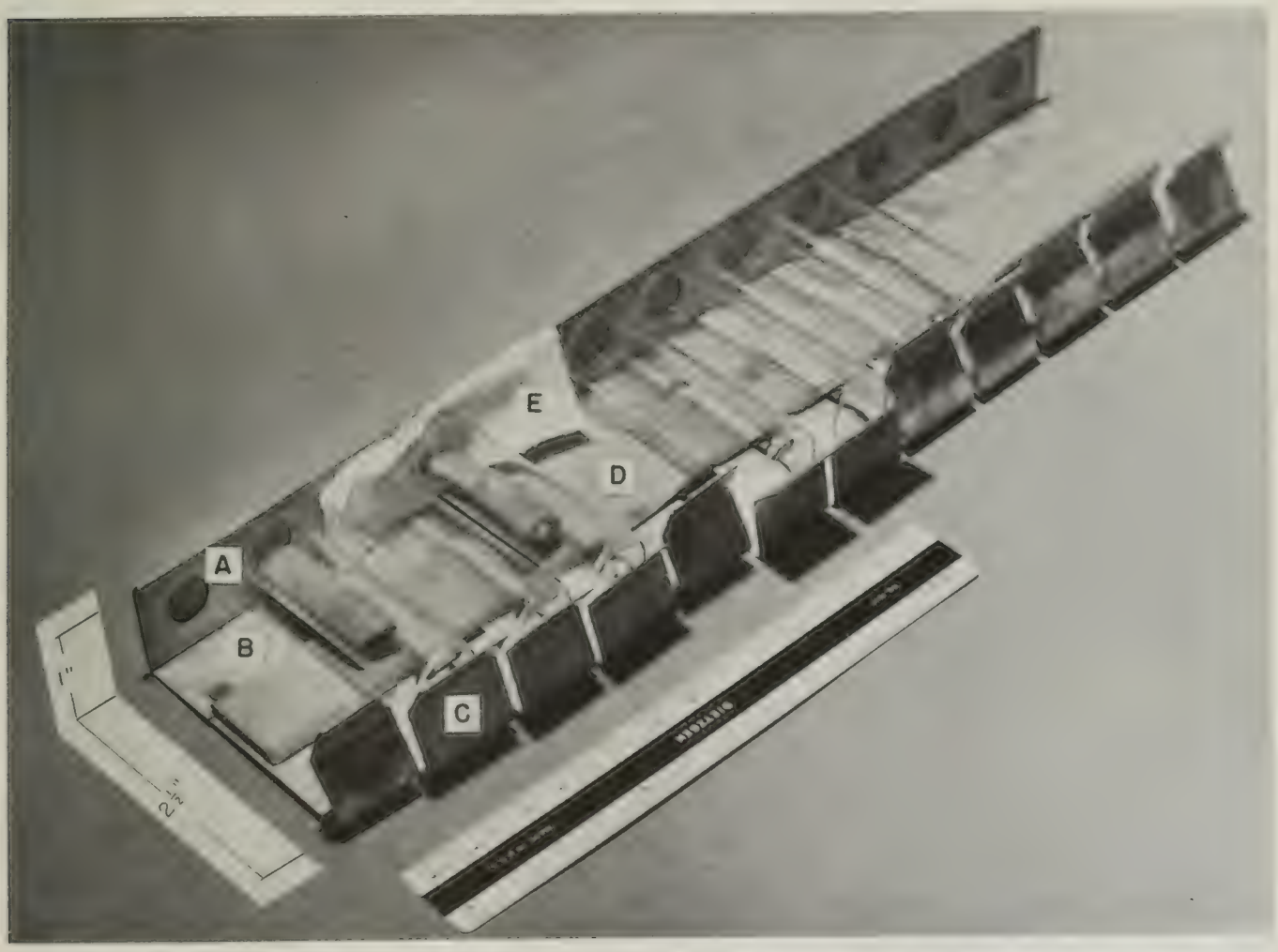

Fig. 5.—Metal clamp rack (28-gauge galvanized sheet iron) designed to hold vials for the rearing of corn borer and other larvae in individual confinement. Part *A* is 1 inch wide; it consists of two thicknesses of metal, one folded against the other. Holes are drilled through the two thicknesses; each hole is one-half inch in diameter and is one-half inch distant from the hole or holes nearest it. A strip of 50-mesh screen is inserted between the two thicknesses of metal before part *A* is bent to form a right angle with part *B*, which is 2-1/2 inches wide. The surface from which clamp springs (one denoted by *C*) are fashioned is 1-1/4 inches wide; the metal is sawed at 1-inch intervals and is bent to form the springs, each one of which faces a hole in part *A*. A strip of wood, *D*, is riveted to part *B* to aid in lining up the mouth of each vial with a hole in part *A*. If lips of the vials are so uneven as to allow escape of young larvae, a 1-inch-wide strip of cellucotton, *E*, is placed between the lips and part *A*. A rack of the size illustrated accommodates vials of 17 x 60 mm., 19 x 65 mm., and 20 x 68 mm.

A special metal clamp rack was therefore designed to hold the vials. With this device, the handling of large numbers of individually reared larvae could be carried out with ease and efficiency. The rack could be made in various lengths to suit individual requirements. It was found that racks accommodating 10 or 12 vials were the easiest to handle. The racks were fashioned of 28-gauge galvanized sheet iron (stainless steel would have been more suitable) bent to the shape shown in fig. 5. When a vial was to be placed in or removed from the rack, pressure was applied to the top of the clamp spring that held it in place.

This clamp rack had several advantages. It made possible the storage of vials in any desired position. All the vials could be checked at a glance. Plugging the mouths of vials with cotton in the usual manner was completely eliminated. The formation of condensed moisture inside the vials was reduced or eliminated by air circulation through the screens.

TESTS WITH INSECTICIDES AND PATHOGENS

With the development of satisfactory laboratory methods for the mass rearing of European corn borer larvae, it became possible to develop techniques for special experimental work. The following received attention: (a) application of insecticides and pathogens to food or substrate; (b) reactions of larvae during periods of exposure to insecticides or pathogens; and (c) estimation of amounts of insecticides or pathogens ingested.

For most of the laboratory experiments with insecticides and pathogens, the simplest and easiest method of feeding the corn borer larvae involved use of only the pericarps of the string beans. The pods were split lengthwise along the sutures, the seeds were removed, and the halves were cut to suitable lengths. The split bean pieces could easily be dusted or sprayed with a solution or suspension, as well as dipped. As a sticker and spreader, 0.1-0.3 per cent methylcellulose (Methocel) was found to give very satisfactory results. The bean pericarps offered large surfaces both for deposit of insecticide or pathogen and for feeding.

The conditions prevailing in the field on a corn leaf could be reproduced to a rather fair degree by using the floating leaf technique. A piece about 3 inches square was cut from the distal end of a leaf of field corn and a hole was punched in the center near the midrib, fig. 6. The distal end was preferred to the proximal end because it tended to be flat, rather than ruffled. If

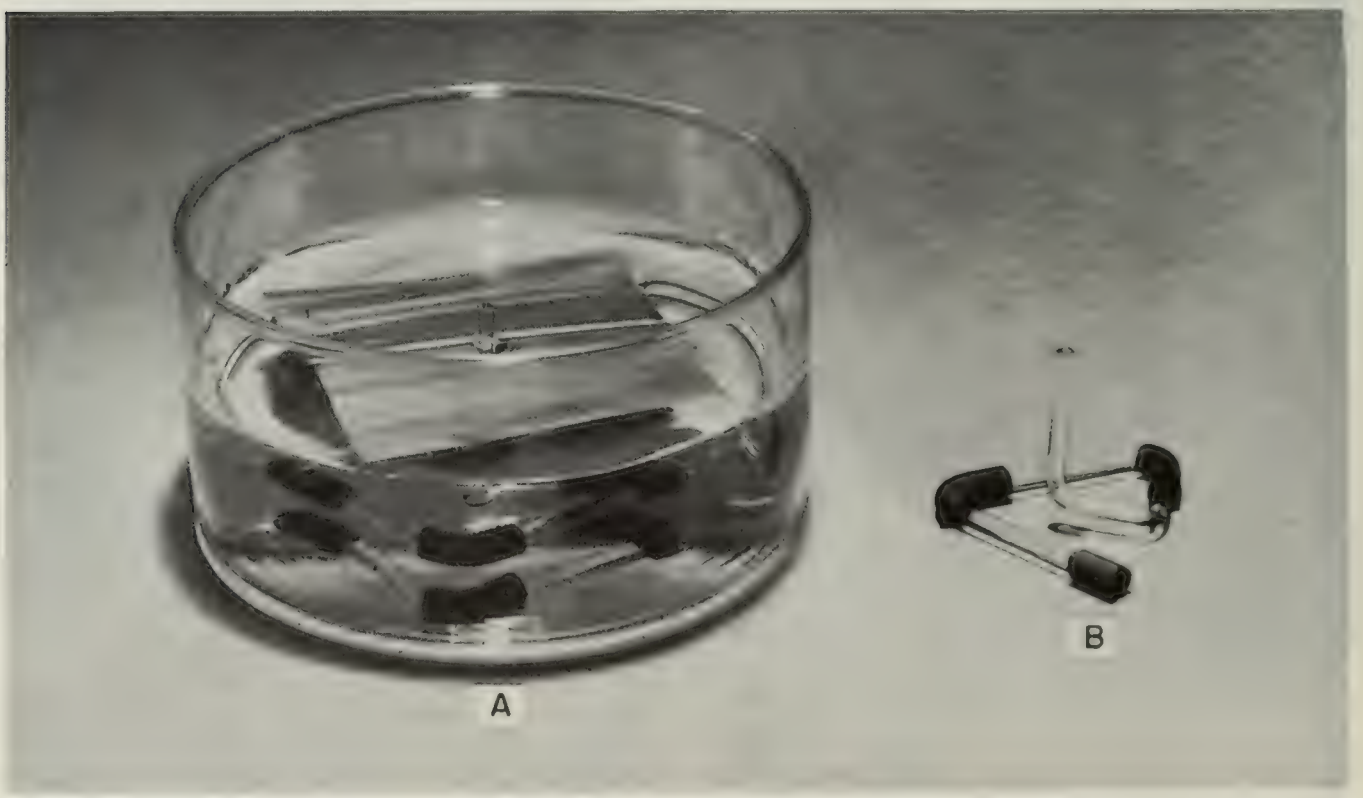

Fig. 6. – Arrangement, *A*, for testing insecticide deposits on corn leaf with the floating leaf technique. A piece of corn leaf is floated on water in a crystallizing dish and kept in place with a triangular-based anchor, *B*, fashioned from a glass rod. The perpendicular part of the anchor is inserted through a hole in the center of the corn leaf. With the help of this anchor, the leaf can be lifted from the water and replaced in it.

carefully placed on the water surface, the leaf floated; it remained fresh for about a week. Some first instar corn borer larvae transferred to a leaf tended to wander around and to come in contact with the water surrounding the leaf. In order to prevent the water from being drawn onto the surface of the leaf following contact with the water by the larvae, a thin film of petroleum jelly was applied around the edges of the leaf and around the hole in the center of the leaf. It was found advisable to treat the leaf on the underside as well. In order to prevent flooding of the upper surface of the leaf when it had been treated with wetting agents, the gutter formed by the midrib was closed at both ends with beeswax. If it was necessary to increase the bouyancy of the leaf, a piece of wax paper of somewhat smaller size was floated beneath the leaf. The leaf was placed in a crystallizing dish of such size that there would be clearance of at least an inch between the leaf and the wall of the dish. The floating leaf was held in the center of the dish by a simple, triangle-shaped anchor made of a bent glass rod. Around this anchor the leaf could turn freely, provided the hole was made large enough. With the help of this anchor the leaf could be lifted out from the water for examination and replaced at any time.

It was sometimes necessary to cover the dish in order to reduce the evaporation of water. Glass or other

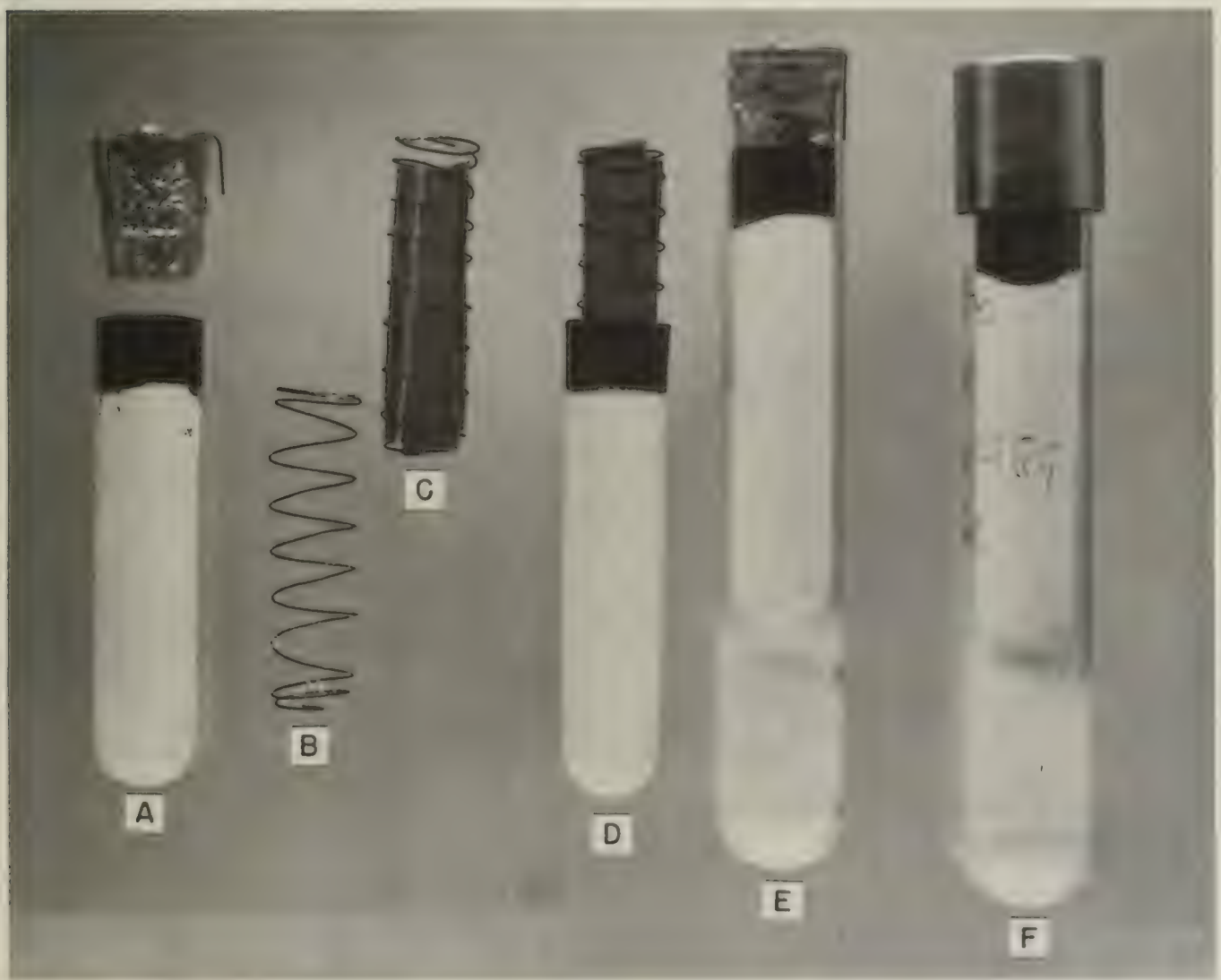

Fig. 7. – Arrangement designed to approximate the conditions prevailing in the whorl of a young corn plant. A 20 x 90 mm. double strength extraction thimble, *A*, is reinforced with a band of lacquer around the lip and two vertical strips of lacquer on opposite sides; also, it is provided with a cork that has been softened and wrapped in tinfoil. A coil of wire, *B*, about 2-1/4 inches long, narrow enough to fit easily in the extraction thimble, and with the lower end somewhat constricted, is used as a holder for a rolled piece of corn leaf, *C*, and inserted, *D*, in the extraction thimble. The thimble is suspended by the cork stopper in a 25 x 150 mm. culture tube, *E*. In the bottom of the tube is an inch of water or saturated salt solution, if either is necessary to maintain the humidity at a specific level. The culture tube is covered, *F*, with an aluminum cap.

water-impermeable covers were not used for this purpose, for they would have promoted the formation of condensed water on the leaf. Covers or caps made from textile, cardboard, or paper, including filter paper, proved satisfactory.

An insecticide or insect pathogen could be sprayed or dusted on the surface of the corn leaf either before or after the edges had been treated. In tests involving insecticides or pathogens, 10 first instar borers were placed, with the help of a wet camel's-hair brush, on the floating leaf or preferably on the top of the glass rod. Diffused light or darkness prevented phototropic responses in the young larvae and helped to reduce the length of a period of wandering during which larvae occasionally got into the water. The length of this period of wandering was reduced also by pollen on the leaf. If pollen was not available, finely ground corn meal, 80-100 mesh, served the purpose. Pollen or corn meal was deposited on the leaf with a small sieve. Small pieces of thin polyethylene film (freezer bag material) or tissue paper placed on the leaf over the larvae appeared to satisfy the thigmotactic responses of the young larvae.

Most of the larvae started to feed in about 6 to 24 hours. Their behavior upon treatment was easily observed. When fast-acting insecticides or pathogens were used, the larvae fed for only a short time and then wandered around aimlessly until eventually they drowned in the water. The number of drowned larvae served as an indication of the effectiveness of the treatment. In untreated checks or in tests where slow-acting ingredients were used, larvae could be reared on floating leaves until the second instar.

The conditions prevailing in the whorl of a young corn plant could, with fair success, be duplicated by still another experimental device. For this device, 20 x 90 mm. double-strength cellulose extraction thimbles were selected. Each extraction thimble, fig. 7, was reinforced with lacquer to preserve its original shape when it was exposed to high humidities. The thimble was closed with a softened cork stopper wrapped in tinfoil.

As a support for the corn leaf, a coil of about 10 turns was made from enameled copper wire. (Stainless steel or aluminum wire would have been preferable.) The coil was about 2-1/4 inches long, constricted at the lower end, and of such diameter that it would fit loosely into the thimble. One or two pieces of corn leaf cut transversely into 4-inch lengths were rolled up and slipped inside the coil. The coil was then inserted into the thimble, first instar corn borer larvae were transferred to the corn leaf, and the mouth of the thimble was closed with a cork stopper. The thimble was then suspended by its cork stopper and a wire hook in a 25 x 150 mm. culture tube. In the bottom of this culture tube, a 1-inch level of water or saturated salt solution was kept in order to help maintain the required degree of humidity. The culture tube was covered with an aluminum cap.

It was found that almost no condensed moisture accumulated inside the extraction thimble and that the corn leaf remained fresh up to 8 days. The wire coil prevented the leaf from touching the side of the thimble. The corn borer larvae fed on the leaf in such a way as to make characteristic feeding patterns. They could be reared in this manner to at least the third instar. When the time arrived for checking the larvae, the coil was lifted out of the thimble, and the leaf was removed from the coil and unrolled. This type of handling apparently did not disturb the larvae.

Each leaf could be treated with an insecticide or pathogen on one or both surfaces before it was placed in the coil. By using either the floating leaf technique or the extraction thimble technique, it was possible to estimate the area of leaf surface, and thus the amount of active ingredient, consumed by young larvae, which tended to remain in the same place for a considerable time after they had started to feed. The area of feeding could be measured by the grid method or by a planimeter, if the pressed and dried leaf displaying the feeding marks was placed in a photographic enlarger and the image was enlarged to a suitable size. The leaf itself could be filed in an envelope.

ADAPTATIONS OF CONTINUOUS MASS REARING TECHNIQUE

The method of rearing European corn borer larvae and the experimental techniques described in the preceding paragraphs were designed to satisfy primarily the requirements of research in the field of biological control. Both the corn borers reared by this method and the techniques described might have wider use.

Since it was found possible to rear 8 to 10 successive generations of the European corn borer in the laboratory under controlled conditions, this insect might be used as a standard experimental subject.

The split bean technique of testing insecticides might be adopted for use in a study of systemic insecticides. If a systemic insecticide is applied to the foliage or stems of a bean plant grown in a greenhouse, or to the soil in which the plant is grown, the amount of translocated insecticide in the pods might be evaluated with a fair degree of accuracy by subjecting the pods to feeding by corn borer larvae.

As it is easy to rear nymphs or even adults of the potato leafhopper, *Empoasca fabae* (Harr.) on whole or sectioned green bean pods, experiments could be designed to test an insecticide against a chewing insect

and a sucking insect at the same time by using the split bean technique.

Nutritional and other physiological studies might also profit from use of the split bean technique if the bean plant is grown in a medium containing nutrients labeled with radioactive isotopes.

For studies with systemic compounds, the floating leaf technique might be employed with known amounts of the compounds added to the water. Not only the mortality of the larvae but also their reactions could be observed. The extraction thimble technique could be adapted for experiments with granulated compounds by depositing the granules between the rolled leaves.

Some of the rearing and research techniques described above have with certain modifications been used in work involving lepidopterous larvae other than those of the corn borer. The experience gained while working with them probably deserves a brief mention.

Larvae of the corn earworm, *Heliothis zea* (Boddie), were sometimes reared in great numbers in the laboratory. Because of their cannibalistic habits, the larvae had to be kept in individual confinement. Vials of 19 x 65 mm. size were found to be suitable for rearing the larvae. The vials were stored either in metal clamp racks, fig. 5, or in wooden racks. It was advisable, at the time the larvae were in the first instar, to seal the orifice of each vial in the wooden racks with a piece of tinfoil, 2 x 2 inches in size, in order to prevent escape of the larvae. Subsequently, aluminum caps, designed for 18 mm. bacterial culture tubes, were used instead of conventional cotton plugs. The formation of excessive amounts of condensed moisture, which could be very bothersome, was prevented by providing each aluminum cap with a seven-sixteenths-inch hole. Then the hole was plugged with a piece of dental cotton wick. For food, pieces of green beans were used exclusively and with very good results.

Larvae of the variegated cutworm, *Peridroma margaritosa* (Haw.), were reared in similar manner. The larvae were, at times, reluctant to accept whole bean pods. However, if the beans were split, the larvae fed on them and developed normally.

Because of the gregarious habits of larvae of the armyworm, *Pseudaletia unipuncta* (Haw.), the mass rearing of this insect usually did not involve many difficulties. When armyworms had to be reared individually, the maintaining of proper humidity was of primary significance. This was achieved very simply by pouring a layer of 4 per cent agar, 10 mm. deep, into the bottom of a 14 x 75 mm. vial, a size which was found to be the most satisfactory for this purpose. The surface of the agar had to be dried for about 12 hours to prevent the larvae from sticking to the surface. The young larvae were fed on oat leaves at first and later on corn leaves.

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